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Nicotinamide and methionine reduce the liver toxic effect of methotrexate

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Abstract

Methotrexate is widely used as a therapeutic agent in different diseases. This therapy is connected with various side effects, including liver toxicity. We have developed a mouse model to demonstrate the toxic effects of methotrexate: mice were given 50 mg/kg acetaminophen, which itself has no effect on the liver. If, additionally, methotrexate is applied, there is an increase in the death rate, as well as in glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) activities. If methotrexate is administered in conjunction with either nicotinamide or methionine, the rise in the death rate and in GOT and GPT activities associated with methotrexate application is markedly reduced. On the basis of these results, it can be concluded that methotrexate therapy should be combined with either nicotinamide or methionine, respectively.

Keywords: Methotrexate; Liver toxicity; Acetaminophen; Nicotinamide; Methionine; Liver protection

Methotrexate (MTX) is widely used in tumor therapy, as well as in the treatment of rheumatoid arthritis (Cash and Knippel, 1994; Guhner et al., 1951; Krall et al., 1989; Pincus et al., 1992; Rothenberg et al., 1988; Scully et al., 1991; Kremer and Phelps, 1992). As many as 0.3% of the patients treated with MTX suffer from severe side effects. The most common side effect is gastrointestinal intolerance. Approximately 50% of patients taking MTX will experience symptoms that range from mild abdominal pain and diarrhea to more-severe nausea and vomiting. MTX-related hepatic toxicity may result in elevated liver function test values, which have been correlated with serious liver disorder (Kremer et al., 1994; Carrol et al., 1994).

In experiments already published, we showed that the hepatotoxic effects of acetaminophen could be prevented by nicotinamide, *N*-acetylcysteine and methionine (Kröger et al., 1996a, 1996b, 1997). This prevention is considered to be due to interference with the metabolisms of oxygen radicals and of nicotinamide-adenine dinucleotide (poly)adenosine diphosphate ribose [NAD-poly(ADPR)].

In the present publication, we have tested whether MTX-induced lesions of the liver could be prevented. We developed a system using low amounts of MTX and acetaminophen (PAR), which induce damage of the liver. Using this system, we could show that nicotinamide and methionine prevent the toxic effect.

1. Materials and methods

All reagents, unless indicated otherwise, were purchased from Sigma-Aldrich Chemie GmbH (Deisenhofen, Germany).

1.1. Animals

Male DBA/1 X B.10(4R) mice, weighing 25–30 g, were housed under standard laboratory conditions and given a standard diet. Acetaminophen was applied by gavage. All other substances were injected intraperitoneally. At the end of the experiments, EDTA-blood was obtained. Any animals that died were taken out of the experiment.

1.2. Enzyme determinations

Glutamate-pyruvate (GPT: EC 2.6.1.2) and glutamate-oxaloacetate transaminase (GOT; EC 2.6.1.1) ac-

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Table 1
Toxicity in mice after a single application of increasing amounts of MTX

Treatment	GOT	GPT
NaCl	52.06 ± 15.03 (11)	45.46 ± 11.42 (11)
MTX, 7.5 mg/kg	56.77 ± 15.81 (8)	44.36 ± 15.03 (7)
MTX, 15 mg/kg	48.01 ± 14.57 (16)	42.51 ± 21.68 (16)
MTX, 25 mg/kg	49.91 ± 12.73 (8)	36.11 ± 7.81 (8)
MTX, 50 mg/kg	57.18 ± 27.04 (12)	46.16 ± 22.85 (12)
MTX, 75 mg/kg	57.34 ± 19.15 (8)	41.48 ± 16.01 (8)
MTX, 100 mg/kg	60.51 ± 13.35 (8)	48.24 ± 5.34 (8)
MTX, 150 mg/kg	60.74 ± 42.45 (8)	48.15 ± 26.35 (8)
MTX, 200 mg/kg	46.59 ± 7.26 (8)	45.52 ± 31.93 (8)
MTX, 300 mg/kg	68.97* ± 19.46 (8)	52.63 ± 19.10 (8)
MTX, 400 mg/kg	64.27 ± 33.44 (6)	37.45 ± 8.60 (6)

Note: MTX and NaCl were given once. Before the application of MTX, the animals were starved for 18 h. Blood was taken 16 h after the application of MTX. Number of animals in parentheses.
* Significance level: p = 0.05.

Table 3
Toxicity in mice after application of MTX and acetaminophen

Treatment	GOT	GPT
NaCl	55.60 ± 9.76 (8)	54.70 ± 11.16 (8)
PAR, 50 mg/kg	43.86 ± 11.22 (8)	46.19 ± 16.95 (8)
PAR, 50 mg/kg, + MTX, 25 mg/kg	79.61* ± 32.69 (10)	56.30 ± 13.80 (10)
PAR, 50 mg/kg, + MTX, 50 mg/kg	144.20* ± 85.55 (9)	74.72* ± 28.01 (9)
PAR, 50 mg/kg, + MTX, 100 mg/kg	233.19* ± 160.61 (7)	159.66* ± 167.80 (7)
PAR, 100 mg/kg	40.04 ± 9.39 (8)	34.17 ± 5.39 (8)
PAR, 100 mg/kg, + MTX, 25 mg/kg	81.96* ± 50.03 (8)	97.69* ± 99.93 (8)

Note: MTX and NaCl were given intraperitoneally over 4 days. Then 3 days later, the animals were starved for 18 h. After this period, MTX as well as NaCl were again applied intraperitoneally. Additionally, the animals recievied acetaminophen (PAR) by gavage. Then 16 h later, blood was taken. Number of animals in parentheses.
* Significance level: p = 0.05.

tivities were determined photometrically according to Bergmeyer (1974). In animals that died in the course of the experiment, enzyme levels were not determined.

1.3. Statistical analysis

All data were analyzed with the Mann-Whitney test as a nonparametric two-sided test (Graf et al., 1987), and the significance level p = 0.05 was determined by using the statistical Wilcox computer program, as indicated by an asterisk.

2. Results

2.1. Influence of increasing amounts of MTX (given once) on toxicity in mice

MTX was applied in increasing concentrations after 18-h starvation of the animals. Sixteen hours later, the animals were killed. No drastic change in the activities of GOT and GPT was observed (Table 1). Even a dosage of 400 mg/kg was without any effect.

2.2. Influence of increasing amounts of MTX (given five times) on toxicity in mice

If MTX is applied five times, toxicity can be seen (Table 2). On application of 50 mg/kg MTX, there is a distinct difference to the reaction in the controls. Even though on application of 100 mg/kg MTX the enzyme activity rises distinctly, the difference in controls is statistically not significant. This finding is due to the small number of experimental animals as well as to the degree of fluctuation between individual animals. It is not possible to explain the cause of death of some experimental animals (Tables 2 and 3).

2.3. Influence of MTX and acetaminophen on toxicity in mice

In earlier publications, acetaminophen given in a concentration of 500 mg/kg by gavage was shown to lead to an increase in GOT and GPT activities (Kröger et al., 1996a, 1996b, 1997). We have combined the use of acetaminophen at a lower dose with MTX. With this combination, there is an increase in GOT and GPT activities (Table 3).

2.4. Influence of MTX, acetaminophen, and methionine on toxicity in mice

When animals are treated with 100 mg/kg of methionine, the GOT and GPT activities remain unchanged

Table 2
Toxicity in mice after several applications of different amounts of MTX

Treatment	Remarks	GOT	GPT
NaCl		55.60 ± 9.76 (8)	54.84 ± 10.99 (8)
MTX, 25 mg/kg		47.35 ± 18.25 (11)	34.74* ± 11.96 (11)
MTX, 50 mg/kg	One animal died	76.40* ± 15.75 (8)	71.73* ± 16.68 (7)
MTX, 100 mg/kg	Seven animals died	292.33 ± 518.64 (6)	396.88 ± 590.07 (6)
MTX, 200 mg/kg	Eight animals died	105.90 ± 0.02 (2)	52.10 ± 0.12 (2)

Note: MTX and NaCl were given over 4 days; then the animals starved for 18 h. After this period, these substances were injected once again; 16 h later, blood was taken. Number of animals in parentheses.
* Significance level: p = 0.05.

Table 4
Toxicity in mice after application of MTX, acetaminophen, and methionine

Treatment	GOT	GPT
NaCl	55.37 ± 12.22 (8)	46.02 ± 17.50 (8)
Methionine, 100 mg/kg	60.78 ± 10.63 (5)	42.26 ± 3.63 (5)
PAR, 50 mg/kg, + MTX, 50 mg/kg	163.18 ± 101.86 (6)	79.47 ± 33.17 (6)
PAR, 50 mg/kg, + MTX, 50 mg/kg, + methionine, 100 mg/kg	80.36 ± 23.66 (5)	66.68 ± 25.55 (5)
PAR, 50 mg/kg, + MTX, 100 mg/kg	292.25 ± 191.47 (4)	168.82 ± 204.13 (4)
PAR, 50 mg/kg, + MTX, 100 mg/kg, + methionine, 100 mg/kg	90.84 ± 16.11 (5)	73.28 ± 48.48 (5)

Note: MTX, NaCl and NA were applied intraperitoneally over 4 days. Then 3 days later, the animals were starved for 18 h. After this period, MTX, NaCl, and NA were given intraperitoneally again. Additionally, the animals received acetaminophen (PAR) by gavage. Then 16 h later, blood was taken. Number of animals in parentheses.

(Table 4). Methionine is nevertheless capable of counteracting (i.e., reducing) the increase in the activity of these enzymes due to MTX and (PAR). This counteraction also applies when a higher dose of MTX is used.

2.5. Influence of MTX, acetaminophen, and nicotinamide on toxicity in mice

In a further experiment, three of six animals died (Table 5). When 50 mg/kg of nicotinamide (NA) was applied, mortality was reduced to zero. With increasing NA doses, there was a reduction in GOT and GPT activities.

Table 5
Toxicity in mice after application of MTX, acetaminophen, and NA, respectively

Treatment	GOT	GPT
NaCl	50.23 ± 9.69 (6)	34.25 ± 6.44 (6)
MTX, 50 mg/kg	69.75 ± 14.76 (6)	47.85 ± 11.90 (6)
MTX, 50 mg/kg, + PAR, 50 mg/kg[a]	106.17 ± 9.47 (3)	65.23 ± 13.57 (3)
MTX, 50 mg/kg, + PAR, 50 mg/kg, + NA, 50 mg/kg	187.12 ± 94.37 (6)	83.82 ± 31.40 (6)
MTX, 50 mg/kg, + PAR, 50 mg/kg, + NA, 100 mg/kg	95.48 ± 34.38 (6)	69.48 ± 25.59 (6)
MTX, 50 mg/kg, + PAR, 50 mg/kg, + NA, 250 mg/kg	97.50 ± 64.90 (5)	64.24 ± 24.04 (5)

Note: MTX, NaCl, and NA were applied intraperitoneally over 4 days. Then the animals were starved for 18 h. After this period, MTX, NaCl, and NA were given again. Additionally, the animals received acetaminophen (PAR) by gavage. Blood was taken 16 h later. Number of animals in parentheses.

[a] Three animals died.

3. Discussion

In the course of therapy with MTX, several toxic effects can arise, including those observed in the liver (Kremer et al., 1994; Carrol et al., 1994). In already published experiments, we showed that oxygen radicals and NAD-poly(ADPR) metabolism play an important role in the genesis of MTX-induced liver toxicity (Kröger et al., 1996a, 1996b, 1997). This finding allows the conclusion that substances interfering with these metabolic pathways (e.g., nicotinamide, *N*-acetylcysteine, methionine) could be used to prevent liver toxic effects of MTX during therapy. In the present paper, a mouse model was established, in which MTX toxicity can be demonstrated. In this system, NA as well as methionine were shown to have an inhibitory effect on MTX-induced liver toxicity. This finding means that MTX leads to liver toxicity through an already described cascade mechanism (Kröger et al., 1999). From these data, one can conclude that therapy with MTX should be combined with the simultaneous administration of either nicotinamide or methionine or both together.

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